Immunofluorescence

Fixing cells

1. Grow cells on slides for 2-4 hours. Incubation time and condition vary depending on cell type. Some cells require at least 4 hours incubation on gelatinised slides in a humid chamber.

2. Rinse slides briefly in PBS to remove unattached cells.

3. Fix cells in 2% PFA at room temperature for 15 minutes. (2.15ml FA + 37.85ml PBS)

4. Wash slides in PBS for 3 times, 5 minutes each. (Can store slides at 4°C overnight at this stage, but only do so when really necessary. Always best to proceed directly to step 5.)

5. Permeabilise cells in 0.4% triton at room temperature for 5 minutes.
   (0.4% triton = 1.6ml 10% triton + 38.4ml PBS)

6. Wash slides in PBS for 3 times, 5 minutes each.

7. Store slides in PBS at 4°C.

Detection

1. Dilute 1st and 2nd layer antibodies in fish gelatine with 5% goat serum.
   (Fish gelatine: 1g liquid stock + 400ml PBS + 100ml water.)

2. Block unspecific antibody-binding by incubating slide in fish gelatine for 3x 5 minutes at room temperature.

3. Incubate slides in 1st layer antibody at room temperature for 2 hours.

4. Wash slides in fish gelatin for 3 times, 3 minutes each, followed by PBS (also 3 times, 3 minutes each).

5. Incubate slides in 2nd layer antibody at room temperature for 1 hour in dark.

6. Wash slides in fish gelatine for 2 times, 3 minutes each, followed by one PBS rinse.

7. Optional: post-fix slides in 2% PFA for 10 minutes at room temperature. Can re-use the PFA used in fixing cells (if the solution had been prepared on the same day).

8. Mount slides with Vectashield (10-15 μl antifade mounting medium ["keep" fluorescence], Vector labs, H-1000), containing 0.1 μg/ml DAPI (from a 1000 x stock of DAPI already in Vectashield at 0.1 mg/ml). Store slides at 4°C.